Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Engineering 42 (2012) 1682 – 1691

Procedia Engineering

www.elsevier.com/locate/procedia

20th International Congress of Chemical and Process Engineering CHISA 2012
25 – 29 August 2012, Prague, Czech Republic

Solute-matrix and solute-solute interactions during supercritical fluid extraction of sea buckthorn leaves

M. Sajfrtova a*, H. Sovova

Institute of Chemical Process Fundamentals of the ASCR, v.v.i., Rozvojová 135, 16502 Prague, Czech Republic

Abstract

In this work, the SFE was applied to extract selected medicinal substances from sea buckthorn (*Hippophae rhamnoides* L.) leaves at different conditions (pressure 20-28 MPa, temperature 40-60 °C and ethanol concentration in CO_2 0-6.9 wt. %) influencing solvent power of CO_2. Interest was focused on the leaf oleoresin (total extract) and its minor components (fat soluble vitamins and carotenoids). The yield of polar component was still steadily increasing at the moment when the extraction is almost finished as non-polar components are exhausted and the increase in total extract is very low. Out of the operation variables, the ethanol concentration in CO_2 showed the strongest effect on the extraction. The final yields of polar components were increasing with rising ethanol concentration in CO_2. The extraction of non-polar components (vitamins and β-carotene) and polar lutein was synchronised with oleoresin, whereas the extraction of non-polar lycopene, a component strongly affected by entrainer, was not influenced by the extraction of oleoresin. The solute-solute interactions were expressed either as a delay of the initial extraction period for some poorly soluble components against the more soluble ones or, on the contrary, as a synchronous extraction of the components similar in structure.

Keywords: Supercritical fluid extraction; sea buckthom leaves; solute-solute interaction; carotenoids

* Corresponding author. Tel.: +420220390202; fax: +420220920661.
E-mail address: sajfrtova@icpf.cas.cz.

doi:10.1016/j.proeng.2012.07.561

1. Introduction

Natural medicinal substances for nutraceuticals, food, cosmetics and pharmaceuticals become much sought after nowadays. Supercritical fluid extraction with carbon dioxide (SFE) is one of the "green" technologies for their separation. It uses neither toxic solvents nor high temperatures and its energy consumption is lower than that of conventional techniques.

Sea buckthorn (*Hippophae rhamnoides* L.) is a deciduous shrub which fruit has a unique composition with high levels of nutritionally and medicinally important components and a high potential as a dietary and medicinal supplement. The chemical composition and the processing of sea buckthorn berries have been reviewed [1]. As a by-product of sea buckthorn fruit processing, the leaves are a very cheap and easy accessible source of carotenoids, flavonoids, tocopherols, free and esterified sterols, triterpenols, and isoprenols [2-4]. Only few studies are available on their pharmacological effects, in contrary to fruits and seed. Sea buckthorn leaves ethyl alcohol extract was reported to possess antioxidant and immunomodulatory [5], anti-inflammatory [6], anti-stress and adaptogenic activity with no toxicity [7].

While the SFE of lipids from sea buckthorn berries and seeds has been studied by several researchers [8-10] and is carried out on large scale, the technique has not been applied to sea buckthorn leaves, according to our knowledge.

Although oleoresin extraction from various plants is described by many models in the literature, there is no universal procedure to evaluate the extraction course of minor components. The same component can indeed show a different relationship to the matrix and its relationship with other components may vary. This is due to different structural properties and chemical composition of any plant or part thereof. The seeds contain a significant proportion of lipids, the main component of the oleoresin from leaves are cuticular waxes, while the roots are mostly formed by the polysaccharide matrix.

In this study, the evaluation was done by comparing the extraction course of the individual components (α- and γ-tocopherol, vitamin K_1, β-carotene, lycopene and lutein) of sea buckthorn leaves depending on the extraction conditions. Furthermore, attention was focused on the possible interaction of minor components during the extraction and on the correlation of their yields with the total extract (oleoresin). The extraction yields of sea buckthorn leaf oleoresin were measured gravimetrically to describe the course of the extraction. The composition of extract samples was analysed by HPLC. carotene).

2. Experimental part

2.1. Materials

The leaves of sea buckthorn. were harvested from plants grown in the Czech Republic. Leaves were dried at room temperature and grinded in a coffee mill prior to the extraction. The average particle size leaves measured by sieve analysis of the particles after extraction was evaluated as the Sauter mean diameters of 0.17 mm.

Carbon dioxide of purity better than 99.9 % was purchased from Linde Gas. Ethanol for spectroscopy (Lachema Neratovice, CR) was used as entrainer. Antioxidant 2,6-di-tert-butyl-*p*-cresol (BHT) was purchased from Fluka Chemie AG. Methanol and terc-butylmethylether (Chromservis s.r.o., CR) were used as a mobile phase for chemical analysis. Chromatographic standards of β-carotene, lycopene, lutein, α- and γ-tocopherol and vitamin K_1 were purchased from Sigma (Sigma-Aldrich, Steinheim, Germany).

2.2. Supercritical fluid extraction

The SFE apparatus used in this work was described in detail in a previous work [11]. Extraction columns of 150 mL (I.D. 30 mm) volume was filled with 10 g of plant particles placed between layers of glass beads serving as solvent flow distributors. The extractor was immersed in a temperature-controlled water bath. CO_2 was pressurised by compressor NovaSwiss 560.0007 and controlled by the pressure regulator unit NovaSwiss 560.0009 to operating pressure. In experiments with entrainer, the stream of CO_2 was before entering the extractor mixed with ethanol pumped at a constant flow rate by high-pressure pump LCP 4020.3 ECOM, CR. The solution was expanded to ambient pressure in a heated micrometer valve behind the extractor and the extract fractions were collected in empty glass traps at ambient temperature, weighed and stored for chemical analysis. At least five samples were taken in each experimental run to describe the course of extraction. The amount of gaseous solvent leaving the trap was measured using a gas meter.

The carbon dioxide flow rate was adjusted to 0.5 $g.min^{-1}$. The extraction pressure was 20 MPa and 28 MPa, the extraction temperatures were 40 °C and 60 °C and the ethanol concentration in CO_2 varied from 0 to 6.9 wt. %. To protect the components against degradation, the small amount (0.05 g into 100 mL) of commercial antioxidant (BHT) was added into ethanol. Most of experiments were finished after 80 g CO_2.g $leaves^{-1}$ passed through the extraction column.

2.3. HPLC analysis

The HPLC apparatus consisted of chromatograph HP 1090M and by UV-vis DAD detector (Hewlett-Packard, Waldbron, Germany). Analytic column with stationary phase Develosil C30-UG 250x4 mm I.D (Nomura Chemical Co. Ltd., Anada-Cho Seto, Japan) was protected by pre-column of size 10×4 mm I.D with the same stationary phase.

The CO_2 extracts were dissolved in 5 ml of methanol – *tert*-Butyl methyl ether mixture and filtered. Non-linear multistep gradient with mobile phase methanol – *tert*-Butyl methyl ether – ammonium acetate solution (0.2 %) was applied: 12 min 95:0:5 (v/v/v) isocratic; gradient 12 min 80:15:5 (v/v/v) and gradient 6 min 30:65:5 (v/v/v). Chromatographic conditions were as follows: the flow rate of the mobile phase was 1 $mL.min^{-1}$, the ambient temperature was 20-23 ºC, and UV detection was performed at λ = 245 nm (vitamin K_1), 275 nm (lycopene), 295 nm (tocopherols) and 480 nm (lutein, β-carotene).

3. Results and discussion

3.1. Extraction yield

The extraction of fat-soluble vitamins (α- and γ-tocopherol, vitamin K_1) and plant pigments (β-carotene, lutein and lycopene) from sea buckthorn leaves was studied under various conditions. The most represented vitamin in the extracts was α-tocopherol, its average yields were 22 and 35 times higher than the yields of γ-tocopherol and vitamin K_1, respectively. Tocopherols β- and δ- were not found in the leaves. Most experiments were performed at temperature 40 °C and pressure 28 MPa, in which the effect of increasing ethanol concentrations in CO_2 from 0 to 6.9 wt. % was studied. The influence of temperature increase to 60 °C and pressure reduction to 20 MPa was measured at ethanol concentration in CO_2 4.3 wt. %. The experimental conditions and their labeling in graphs are summarized in Table 1.

Table 1. The overview of extraction conditions and the final yields of sea buckthorn leaf oleoresin and its minor components

Experiment Nr./ Mark	1 / ♦	2 / ●	3 / ■	4 / ▲	5 / ◊	6 / Δ
			Extraction conditions			
p [MPa]	28	28	28	28	28	**20**
T [°C]	40	40	40	40	**60**	40
Ethanol in CO_2 [wt. %]	**0**	**1.5**	**4.3**	**6.9**	4.3	4.3
			Yields [mg.g leaves^{-1}]			
Extract	74.6	77.4	80.1	91.1	86.7	88.9
β-Carotene	0.16	0.20	0.18	0.16	0.18	0.14
Lycopene	0.019	0.026	0.040	0.041	0.034	0.036
Lutein	0.025	0.040	0.054	0.063	0.043	0.042
α-Tocopherol	3.12	3.49	2.90	3.09	3.81	3.42
γ-Tocopherol	0.15	0.19	0.18	0.06*	0.21	0.10*
Vitamin K_1	0.085	0.083	0.073	0.094	0.100	0.086

* Too low values probably due to analytical error

As apparent in Table 1, the lowest extraction yield of oleoresin was obtained using pure CO_2. However, the increased concentration of ethanol in CO_2 led to co-extraction of polar components and increase of oleoresin yield from 74.6 to 91.1 mg.g leaves^{-1}. As expected, the amount of ethanol added to CO_2 did not affect the yield of non-polar vitamins. Both increase of temperature to 60 °C and reduction of pressure to 20 MPa led to a slight increase of yields of oleoresin and all vitamins (in case of neglecting the result of γ-tocopherol at experiment Nr. 6). The β-carotene yield was influenced neither by the addition of ethanol in CO_2 nor by the temperature but the reduced pressure of 20 MPa caused a decrease in its yield from 0.18 to 0.14 mg.g leaves^{-1}. The extraction of lycopene was strongly influenced by the ethanol concentration in CO_2 despite its non-polar character. The addition of 6.9 wt. % of ethanol to CO_2 led to doubling the lycopene yield compared to the extraction with pure CO_2. As expected the yield of polar lutein was strongly dependent on the concentration of ethanol in CO_2; its yield with 6.9 wt. % ethanol in in CO_2 was tripled compared to the yield with pure ethanol Reducing the pressure and the temperature increase caused a slight decrease in the yield of lycopene and lutein.

3.2. Component interactions

The correlations of monitored components during the extraction were determined by plotting the yields of compared components obtained in course of the extraction against each other. The dependence of the γ-tocopherol and vitamin K_1 yields on the α-tocopherol yield obtained in course of the extraction (Figure 1) shows that both components are extracted always in a certain ratio to the prevailing α-tocopherol. After removing the failed experiments, it could be possible to interpolate the plotted points by linear line. This correlation is probably given by the similar structure of all three vitamins.

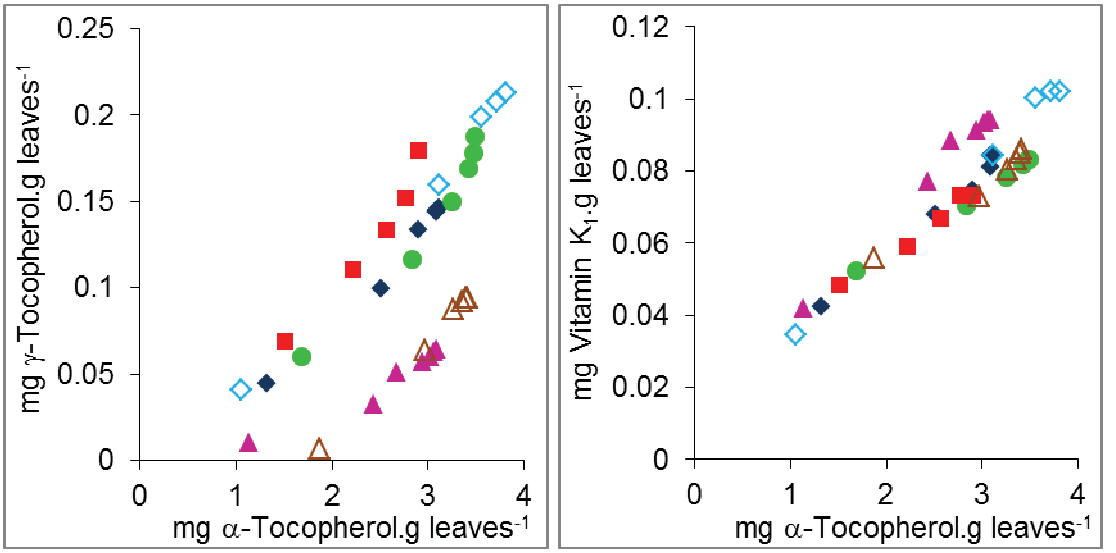

Fig. 1. The correlation of γ-tocopherol and vitamin K_1 with α-tocopherol in course of extraction from sea buckthorn leaves; for meaning of symbols see Table 1

The extraction of carotenoids was slower than the extraction of more soluble tocopherols. It illustrates the Figure 2 where the yields of β-carotene against α-tocopherol are plotted. The yields of carotenoids initially grew very slowly (β-carotene) or not at all (lutein), but after completion of α-tocopherol extraction it started to rise rapidly.

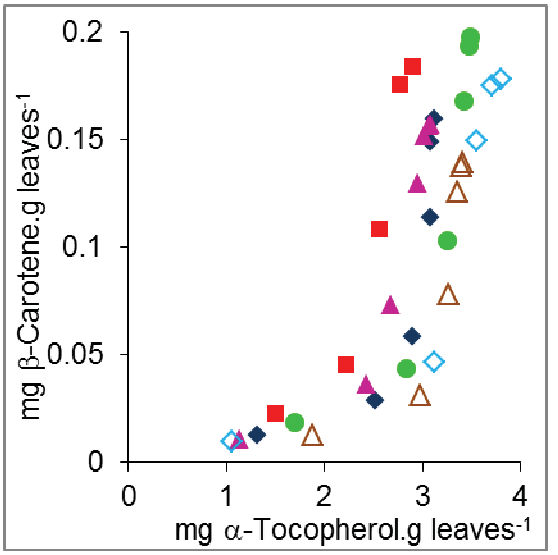

Fig. 2. The correlation of β-carotene with α-tocopherol in course of extraction from sea buckthorn leaves; for meaning of symbols see Table 1

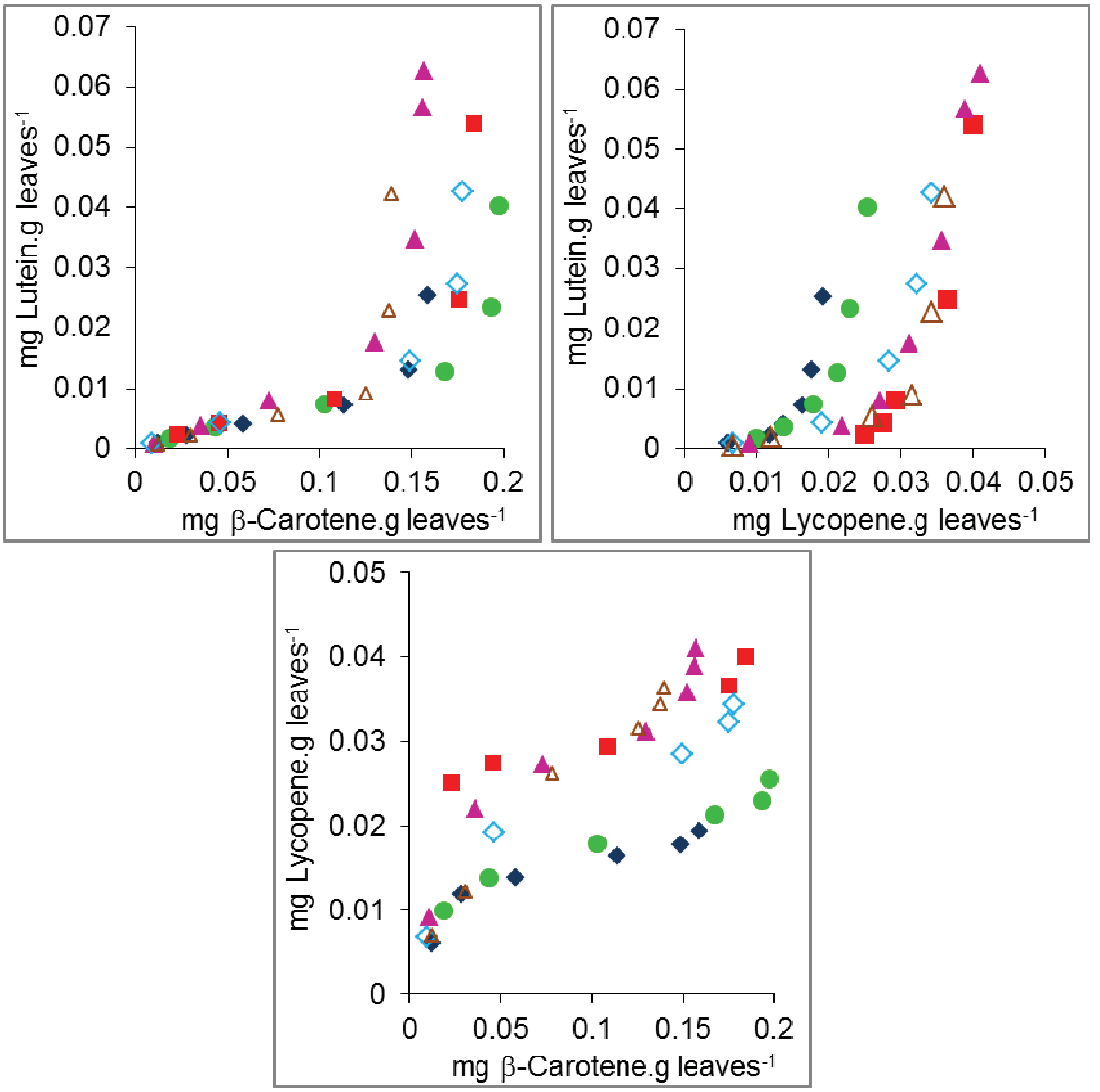

Fig. 3. The correlation of carotenoids in course of extraction from sea buckthorn leaves; for meaning of symbols see Table 1

A similar behavior exhibited β-carotene and lycopene in relation to the less soluble lutein, as shown in Figure 3, where one can also observe the effect of addition of ethanol to CO_2 on the extraction of carotenoids. Lutein was extracted in a certain ratio to β-carotene until β-carotene was present in the plant. Extraction of lycopene was independent of other carotenoids and was affected in particular by the concentrations of ethanol in CO_2.

3.3. Correlation of component with oleoresin

The correlations of monitored components with total extract (oleoresin) during the extraction were determined by plotting the yields of compared components obtained in the course of the extraction against the yield of oleoresin.

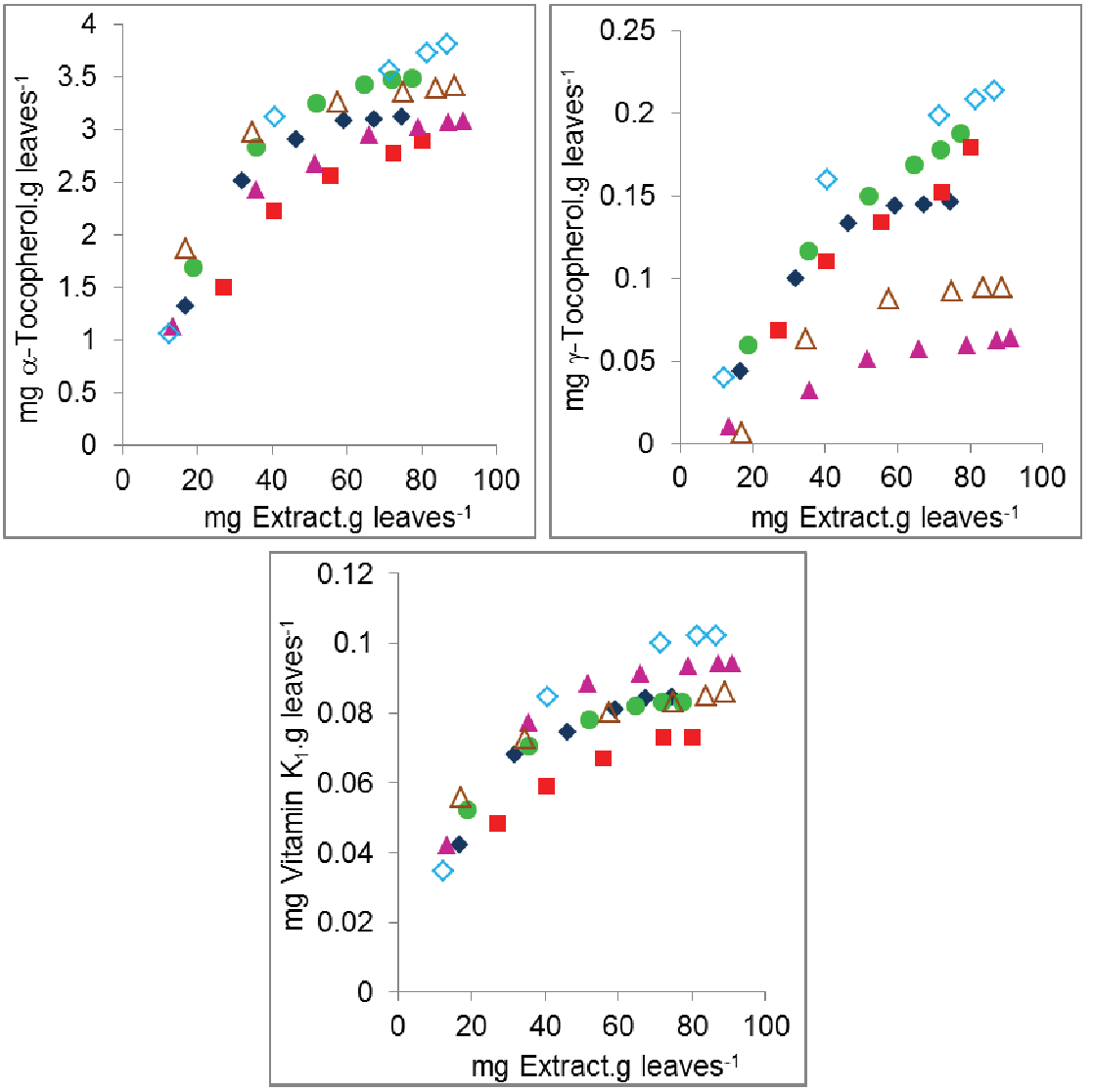

Fig. 4. The correlation of vitamins with total extract in course of extraction from sea buckthorn leaves; for meaning of symbols see Table 1

When the incorrect data of γ-tocopherol are neglected, it can be seen in Figure 4 that the extraction process all vitamins correlated with the oleoresin extraction, especially at the beginning of experiment, when the easily soluble non-polar compounds are extracted. This relationship ceases to be obvious with

the growing yield of oleoresin, since the extraction of these three vitamins is complete when the total extract is rising.

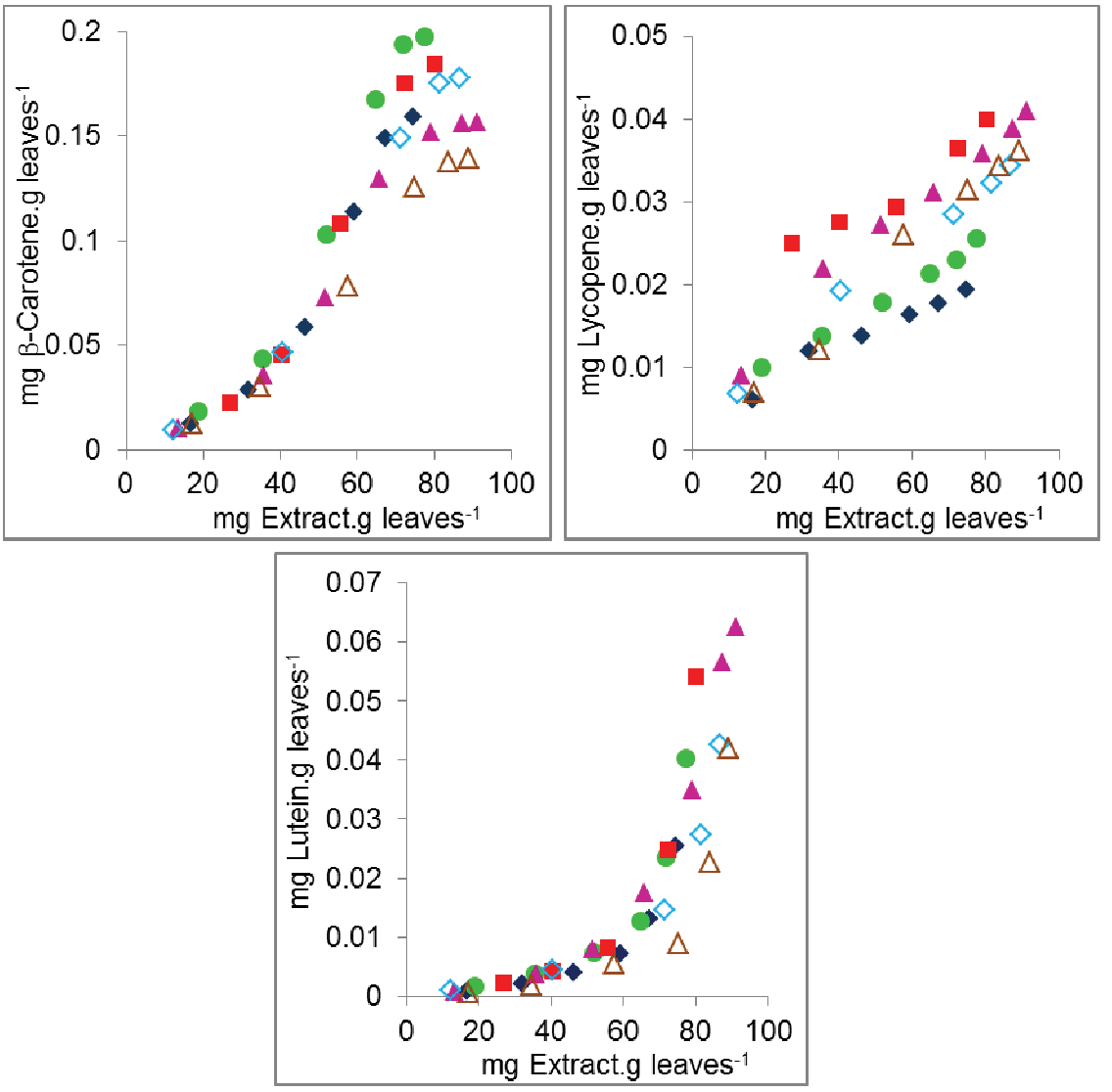

Fig. 5. The correlation of carotenoids with total extract in course of extraction from sea buckthorn leaves; for meaning of symbols see Table 1

The extraction course of individual carotenoids was different, as shown in Figure 5. In contrast to β-carotene and lutein, which yields correlated with the total extract irrespectively of the conditions, the extraction of lycopene was affected by concentration of ethanol in CO_2 and independent on the total extract or other components.

4. Conclusion

The extraction yields of individual components of sea buckthorn leaves were measured at equilibrium conditions to examine the effects of pressure, temperature and ethanol concentration in CO_2 on their final yields as well as the solute-matrix and solute-solute interactions.

The published papers on the SFE from plants are focused on the yield of oleoresin and its composition under different conditions, but data on the extraction kinetics is very limited and mostly concern only oleoresin or one of its components. In this work, however, the extraction rate of more components was examined simultaneously and the main contribution is the description of their interactions and correlation with oleoresin.

An intense increase in the yield of polar component lutein was observed when the extraction of oleoresin seemed to be almost finished. In contrast, the extraction of non-polar vitamins and β-carotene was already complete at this time. Addition of ethanol to CO_2 significantly facilitates the dissolution of all components, but the final yields of non-polar components are not affected. Increasing temperature has a positive effect on the extraction of vitamins and oleoresin.

Extraction of non-polar components (vitamins, β-carotene) and polar lutein proceeded in parallel with oleoresin while the nonpolar lycopene, which shows a strong dependence on the ethanol concentration in CO_2, is affected neither by the extraction of oleoresin nor other components. The extraction course of carotenoids is influenced by the extraction of vitamins, which are extracted simultaneously. A delay in extraction of β-carotene is caused by initial extraction of more soluble α-tocopherol. Similar behavior exhibit also β-carotene and lycopene in relation to the less soluble lutein.

The results extend the knowledge of SFE of medicinal substances from plants and provide information about the most suitable conditions and extraction time for the extraction of given substance, which can be used to control the concentration of certain components in the extract as desired.

Acknowledgements

The financial support by the Technology Agency of the Czech Republic via grant TA01010578 is gratefully acknowledged.

References

[1] Beveridge T, Li TS, Oomah BD, Smith A. Seabuckthorn products: manufacture and composition. *J Agric Food Chem* 1999;**47**:3480-8.

[2] Salenko VL, Kukina TP, Karamyshev VN, Sidelnikov VN, Pentegova VA. Chemical investigation of *Hippophae rhamnoides*. II. Main components of the neutral reaction of the saponification products of an extract of the leaves of the sea buckthorn. *Chem Nat Compd* 1986;**21**:481-5.

[3] Chen C, Liu B, Yu Y. Studies on the pigment of sea buckthorn. *Hippophae* 1995:**7**:34-40.

[4] Goncharova NP, Glushenkova AI.Lipids of the leaves of two forms of central Asian sea buckthorn. *Chem Nat Compd* 1996;**32(4)**:585-6.

[5] Geetha S, Sai RM, Mongia SS, Singh V, Ilavazhagan G, Sawhney RC. Evaluation of antioxidant activity of leaf extract of Seabuckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. *J Ethnopharmacol* 2003;**87**:247-51.

[6] Ganju L, Padwad Y, Singh R, Karan D, Chanda S, Chopra MK. Anti-inflammatory activity of sea buckthorn (*Hippophae rhamnoides*) leaves. *Int Immunopharmcol* 2005;**5**:1675-84.

[7] Saggu S, Diveker HM, Gupta V, Sawhney RC, Banerjee PK, Kumar R. Adaptogenic and safety evaluation of sea buckthorn (*Hippophae rhamnoides*) leaf extract: a dose dependent study. *Food Chem Toxicol* 2007;**45**:609-17.

[8] Cenkowski S, Yakimishen R, Przybylski R, Muir WE. Quality of extracted sea buckthorn seed and pulp oil; *Can Biosyst Eng* 2006;**48(3)**:9-16.

[9] Cossuta D, Simandi B, Hohmann J, Doleschall F, Keve T. Supercritical carbon dioxide extraction of sea buckthorn (Hippophae rhamnoides L.) pomace. *J Sci Food Agri* 2007;**87(13)**:2472-81.

[10] Sajfrtova M, Lickova I, Wimmerova M, Sovova H, Wimmer Z. β-Sitosterol: supercritical carbon dioxide extraction from sea buckthorn (Hippophae rhamnoides L.) seeds. *Int J Mol Sci* 2010;**11**:1842-1850.

[11] Sajfrtova M, Sovova H, Opletal L, Bartlova M. Near-critical extraction of β-sitosterol and scopoletin from stinging nettle roots. J. Supercrit. Fluids. 2005;**35(2)**: 111-8.